36. (New) The plant according to claim 32, wherein the DNA sequence encoding the anti-bacterial peptide from the Diptera insect is operably linked to a signal sequence from a plant gene.

37. (New) The plant according to claim 32, wherein the inducible promoter is a promoter induced by stress.

38. (New) The plant according to claim 37, wherein the promoter induced by stress is a promoter of the tobacco PR-1a gene.

39. (New) The plant according to claim 32, wherein the expression cassette has a terminator of the tobacco PR-1a gene.

40. (New) The plant according to claim 32, wherein the constitutively expressed promoter is the Cauliflower mosaic virus 35S promoter. --

REMARKS

Status of the Application

Claims 21-40 are pending with entry of this amendment, with claims 1-20 being canceled and claims 21-40 being added herein.

Claims 1-3, 5-13, 15, 16, and 18-20 were rejected under 35 U.S.C. §112, first paragraph, as allegedly lacking written description. Claims 1-3, 5-13, 15, 16, and 18-20 were rejected under 35 U.S.C. §112, first paragraph, as allegedly being enabled only for claims limited to a recombinant gene, expression vector, transgenic plant comprising the Sarcotoxin 1a gene. Claim 20 was rejected for being indefinite. Claims 1-5, 10 and 20 were rejected under 35 U.S.C. §102(e) as allegedly being anticipated by Jaynes *et al.* (U.S. Patent 5,597,945). Claims 1, 2 and 20 were rejected under 35 U.S.C. §102(e) as allegedly being anticipated by Broekaert *et al.* (U.S. Patent 5,538,525).

Support for the Amendments

Claims 1-20 are canceled, and claims 21-40 are submitted with this Amendment to further clarify the invention. New claims 21-31 are directed to, *inter alia*, a method of conferring resistance to pathogenic fungi on a plant using an anti-bacterial gene from a Diptera insect. New claims 32-40 are directed to, *inter alia*, a plant comprising an anti-bacterial gene from a Diptera insect, wherein the plant confers resistance to pathogenic fungi. In these claims, an inducible promoter which is operably linked to an anti-bacterial gene is positioned adjacent to a constitutive promoter operably linked to a drug resistance gene.

Support for the new claims can be found throughout the specification, claims, and drawings, as originally filed. For example, support for new claim 21 can be found in, *e.g.*, originally filed claim 1. Support for new claims 22 and 33 can be found in, *e.g.*, originally filed claim 2. Support for new claims 23 and 34 can be found in, *e.g.*, originally filed claim 4. Support for new claim 24 can be found in, *e.g.*, originally filed claim 5 and on page 12, lines 13-16 of the specification. Support for new claims 25 and 35 can be found in, *e.g.*, originally filed claim 6. Support for new claims 26 and 36 can be found in, *e.g.*, originally filed claim 7. Support for new claims 27-29, 37 and 38 can be found in, *e.g.*, originally filed claim 8. Support for new claims 30 and 39 can be found in, *e.g.*, originally filed claim 9. Support for new claim 31 can be found on, *e.g.*, page 12, line 13 of the specification. Support for new claim 32 can be found on, *e.g.*, page 12, lines 4-16 of the specification. Support for new claim 40 can be found in, *e.g.*, originally filed claim 11. No new matter has been introduced.

The Telephone Interview

Applicants' representative greatly appreciates the courtesy shown by Examiner Nelson in the October 18, 1999 telephone interview and further appreciates the Examiner's careful consideration of the arguments made during the interview.

The Rejections under 35 U.S.C. §112, First Paragraph

A. Written Description Rejection

Claims 1-3, 5-13, 15, 16 and 18-20 were rejected under 35 U.S.C. §112, first paragraph, for allegedly lacking an adequate written description. In the Office Action, Examiner continues to assert that the claimed invention lacks an adequate written description,

because the specification only describes fungal resistant transgenic plants comprising the Sarcotoxin 1a gene, and not other fungal resistant transgenic plants. In view of this statement, it appears to be the Examiner's position that the written description requirement set forth in *University of California v. Eli Lilly*, 43 USPQ2d 1398 (Fed. Cir. 1997) is applicable to claims directed to transgenic plants or expression cassettes comprising an anti-bacterial gene as recited in the claims.

Applicants respectfully traverse this rejection for the reasons provided in the previous Amendments. Applicants further request the Examiner's reconsideration for the following reasons.

New claims 21-31 are directed to *methods* of conferring resistance to pathogenic fungi on a plant using a DNA sequence encoding an anti-bacterial peptide from a Diptera insect. The novelty of these claims is not based on the discovery of a new class of nucleic acids (as was the case in *University of California*). Rather, it is based at least in part on the surprising discovery that anti-bacterial genes can be used in a method to confer anti-fungal properties on transgenic plants comprising them. As such, the holding in *University of California* is clearly inapplicable to analysis of these claims.

Similarly, the inventive aspect of claims 32-40 is not based on the discovery of new class of nucleic acids. New claims 32-40 are directed to a plant comprising an anti-bacterial gene from a Diptera insect, wherein the plant confers resistance to pathogenic fungi. The inventive aspect lies in part on the positioning of an inducible promoter and a constitutive promoter in an expression vector and its effect on the anti-bacterial gene expression. Specifically, when an inducible promoter which is operably linked to an anti-bacterial gene is positioned adjacent to a constitutive promoter, the constitutive expression of the anti-bacterial gene is observed under the influence of a constitutive promoter. Moreover, the expression is increased by adding an agent that induces the inducible promoter. *See*, page 11, last line to page 12, line 16 of the specification. As an anti-bacterial gene is not the inventive aspect of claims 32-40, the written description requirement set forth in *University of California* is inapplicable to these claims.

For these reasons, the rejection is improper for the claims as previously presented or currently pending. Withdrawal of the rejection is respectfully requested.

B. Enablement

Claims 1-3, 5-13, 15, 16 and 18-20 were rejected under 35 U.S.C. §112, first paragraph, as allegedly being enabling only for claims limited to a recombinant gene, an expression vector, and a transgenic plant comprising the Sarcotoxin 1a gene. The Examiner alleges that Applicants have not provided any guidance for isolating other anti-bacterial genes or other anti-bacterial genes known in the art which could be used to produce the claimed transgenic plants with fungal resistance. Then the Examiner concludes that undue trial and error experimentation would be required.

Applicants respectfully traverse this rejection. As stated in the previous Amendments, enablement is not precluded by the necessity of some experimentation, such as routine screening. As the Court of Appeals for the Federal Circuit stated: "the key is 'undue', not 'experimentation'" in determining whether pending claims are enabled. *In re Wands*, 8 USPQ2d 1400 (Fed. Cir. 1988). This decision makes clear that a considerable amount of experimentation is permissible if it is merely routine, or if the specification provides a reasonable amount of guidance respect to the direction in which the experimentation should proceed.

Applicants respectfully submit that the Examiner's requirement to limit the claims to Sarcotoxin la is unduly restrictive, because a number of other anti-bacterial peptides from a Diptera insect were well known as of the instant filing date. For example, Wicker *et al.* describes that several families of bacterial peptides have been isolated from immune insects, including, *e.g.*, ceprocins, attacins, dipericins, insect defensins, apidaecins and various homologues referred to as Sarcotoxins and sapecins. (*see, e.g.*, page 22493, first column of *J. Bio. Chem.*, 25:22493-8 (1990), attached with this Amendment). In light of this, those skilled in the art would have been able to introduce into a plant various anti-bacterial genes from a Diptera insect, other than Sarcotoxin la, without undue experimentation.

Applicants also submit that the experimentation necessary to identify a working embodiment of the invention (*i.e.*, an anti-bacterial peptide from a Diptera insect that confers fungal resistance) other than Sarcotoxin 1a is not undue. It is a simple matter to screen for working embodiments of Diptera anti-bacterial genes using the routine screening methods as taught in the present specification. For example, Example 7 of the specification describes *in*

vitro methods for measuring an anti-fungal activity of Diptera anti-bacterial peptides. Specifically, each petri dish of a suitable medium is inoculated with a pathogenic fungi (*e.g.*, Fusarium oxysporum F-3, Rhizoctonia solani, or Rizoctonia solani AG-4) and the size of each hypha growing is measured. A Diptera anti-bacterial peptide that exhibits anti-fungal activity can be screened by identifying a peptide that reduces the fungal growth, *e.g.*, in a concentration dependent manner. Moreover, Examples 8-10 describe methods for measuring anti-fungal activity of an anti-bacterial peptide from a Diptera insect using transgenic plants. For example, as described in Examples 8 and 9, young transgenic plants growing in petri dishes are inoculated by pathogenic fungi. After several days of incubation, green surviving or healthy plants can be identified as resistant plants. Furthermore, as described in Example 10, the size of brown lesions in transgenic plant leaves that are inoculated with pathogenic fungi can be measured to determine whether an anti-bacterial peptide possesses anti-fungal activity. The identification by Applicants of a Diptera anti-bacterial gene that possesses anti-fungal activity (*e.g.*, Sarcotoxin 1a) using routine experimentation as described above provides strong evidence that other anti-bacterial peptide that confers anti-fungal activity can also be identified with the same routine methods.

Identifying Diptera anti-bacterial genes that possess anti-fungal activity does not require anything other than routine cloning and screening of expression products. Such simple screening procedures have never been considered "undue" experimentation by the courts or the Patent Office. Indeed, a rejection of a claim for undue breadth/lack of enablement has always been considered inappropriate when "one of skill could readily determine any one of the claimed embodiments." *See*, MPEP § 2164.08. This standard is further explained in the "Training Materials for Examining Patent Applications with Respect to 35 USC Section 112, First Paragraph-Enablement Chemical/Biotechnological Applications" at sections 3.A.2.b.i.(c). The Office explains that, for instance, "even though a listing of all of the possible DNAs which encode a given protein is a practical impossibility due to the enormous number of such nucleic acids, any *particular* sequence can be written by one of skill given the disclosure and the sequence can be ordered from a company which synthesizes DNA." Similarly, in the present case, any desired nucleic acid encoding a Diptera anti-bacterial sequence, as recited in the claims, can be ordered from a commercial source or created using standard cloning techniques.

These nucleic acids can subsequently be tested for activity using the simple methods taught in the specification. Nothing articulated by the rejection contradicts this clear indication of the ability of one of skill to readily determine the operability of any potential claimed embodiment. Thus the claims, as previously presented or currently pending, are clearly enabled. Accordingly, withdrawal of the rejection is respectfully requested.

The Rejection under 35 U.S.C. §112, Second Paragraph

Claim 20 was rejected under 35 U.S.C. §112, second paragraph, as being indefinite in the recitation of the term "and" instead of "or." Claim 20 has been canceled, and the rejection is now moot.

The Rejections under 35 U.S.C. §102(e)

Claims 1-5, 10 and 20 were rejected under 35 U.S.C. §102(e) as being anticipated by Jaynes *et al.* (U.S. Patent 5,597,945). The Examiner states that "the plants of Jaynes are the same as Applicant in that they are plants transformed with Sarcotoxin 1a gene. Hence, all of the properties of the transgenic plants are an inherent property of the plants of Jaynes." *See* page 5 of the Office Action.

Applicants respectfully traverse this rejection for the reasons provided in the previous Amendments. Applicants further request the Examiner's reconsideration for the following reasons.

Jaynes *et al.* is not an enabling reference and does not anticipate new claims 21-31 directed to methods of conferring resistance to pathogenic fungi on a plant. Jaynes *et al.* prophetically describes transgenic plants with a wide spectrum of microbial resistance and provides no actual results upon which one of skill could reasonably conclude that their transgenic plants possess anti-fungal properties. As noted in the previous Amendments, transgenic plants that comprise an anti-bacterial gene from a Diptera insect are not necessarily resistant to pathogenic fungi. Therefore, it cannot be necessarily assumed that anti-fungal activity is inherent in any transgenic plants comprising an anti-bacterial gene from a Diptera insect, such as those of Jaynes *et al.* In support, Applicants submit Florack *et al.*, *Transgenic Research* 4:132-141 (1995). Florack *et al.* transformed tobacco with an anti-bacterial peptide, Cecropin B, but failed to confer resistance to pathogenic bacteria on the resulting transgenic

plant (*see* the abstract). Thus, the fact that a peptide exhibits anti-bacterial activity *in vitro* does not allow one to reasonably conclude that transgenic plants comprising such a peptide would confer resistance to bacteria, let alone to fungi (which are biologically distinct from bacteria as previously discussed). Similarly, one cannot reasonably conclude that Jaynes *et al.*'s transgenic plants comprising an anti-bacterial gene inherently possess anti-fungal activity. Accordingly, Jaynes *et al.* is not an enabling reference and does not anticipate the claimed invention.

Applicants further note that the plant expression system used by Florack *et al.* was probably not efficient enough to express an amount of the peptide necessary to confer resistance to pathogenic bacteria on the plant. Moreover, based on the teachings of the present specification, resistance to pathogenic fungi would be successfully conferred on a plant by using Cecropin B or other peptides from a Diptera insect.

Jaynes *et al.* also does not anticipate new claims 32-40. Claims 32-40 are directed to a transgenic plant comprising an expression vector comprising a DNA sequence encoding an anti-bacterial peptide from a Diptera insect operably linked to an inducible promoter and a drug resistance gene operably linked to a constitutively expressed promoter, wherein the two promoters are positioned adjacent to each other. Jaynes *et al.* does not teach or suggest a structure of an expression vector wherein an inducible promoter and a constitutively expressed promoter are positioned adjacent to each other. Therefore, Jaynes *et al.* does not teach every element of claims 32-40. Accordingly, withdrawal of the rejection is respectfully requested.

Claims 1, 2 and 20 were rejected under 35 U.S.C. §102(e) as being anticipated by Broekaert *et al.* (U.S. Patent 5,538,525). The Examiner alleges that Broekaert *et al.* describes transgenic plants with fungal resistance.

Applicants respectfully traverse this rejection for the reasons provided in the previous Amendments and the above. While Broekaert *et al.* states that their biocidal proteins show a wide range of anti-fungal activity, Broekaert *et al.* does not test anti-fungal properties of their transgenic plants. As described above, not all transgenic plants comprising anti-bacterial proteins would have anti-fungal properties (*see, e.g.*, Florack *et al.*). As such, one of

skill could not reasonably conclude that Broekaert *et al.*'s transgenic plants possess anti-fungal properties. Therefore, Broekaert *et al.* is not an enabling reference. Accordingly, withdrawal of the rejection is respectfully requested.

Claims 1-20 were rejected under 35 U.S.C. §103(a) as being unpatentable over Jaynes *et al.* (U.S. Patent 5,597,945) in view of Applicants' admission. Claims 1-20 were also rejected under 35 U.S.C. §103(a) as being unpatentable over Broekaert *et al.* (U.S. Patent 5,538,525) in view of Applicants' admission.

Applicants respectfully traverse this rejection for the reasons provided in the previous Amendments. Moreover, Applicants respectfully submit that no "admission" of the invention has been made by Applicants. Page 9, lines 25-33 of the specification (relied upon by the Examiner as "Applicants' admission") states that Shinshi *et al.*, *Plant Mol. Biol.* 14:357-368 (1990) describes a tobacco chitinase protein having a chitin binding region and a catalytic region which regions are joined by a hinge region. There is no teaching or suggestion in Shinshi *et al.* to use the hinge region in an expression vector. Nor did Applicants make any admission that using this hinge region as a linker was known in the prior art. In fact, the only place of record which teaches using the hinge region as a linker is in Applicants' own specification, which the Examiner has improperly used to reject the claims.

At pages 7-8 of the Office Action, the Examiner alleges that

> Applicants has taught no particular purpose for the chitinase hinge region, and hence the inclusion of the hinge region in the vector is considered to be an insignificant, *matter of choice*, and not a nonobvious modification of the prior art references. Although Shinshi has not taught the advantages of the inclusion of the hinge region in an expression vector, neither has Applicant. *See In re Kuhle* 188 USPQ 7 (CCPA 1975), which teaches that the use of a claimed embodiment which solves no apparent problem and provides no unexpected results is deemed an *obvious matter of choice*. (emphasis added).

In view of this statement, it appears to be the Examiner's position that the use of a tobacco chitinase hinge region recited in the present claims is an obvious design choice. Obviousness cannot be established by merely alleging that modifying Jaynes *et al.* or Broekaert *et al.* is a "matter of choice." As specifically addressed by the Board of Appeals

recently, this type of conclusory statement is not a reason why the skilled artisan would have been motivated to arrive at the claimed invention in view of Jaynes *et al.* or Broekaert *et al.* *See, Ex parte Willam R. Garrett*, No. 580-81, 1986 Pat. App. LEXIS 8 (Bd. Pat. App. & Inter. 1986), a copy of which is attached. Since the Examiner has not explained why the skilled artisan would have been motivated to modify the references to arrive at the claimed invention, obviousness has not been established.

During the telephone interview on October 18, 1999, Applicants were advised by the Examiner to submit a declaration discussing the unexpected results obtained with the chitinase hinge region. As explained in the previous Amendments, a showing of unexpected results is not necessary when a *prima facie* case of obviousness has not been established. In the absence of reasoning or evidence to show why one of skill would be motivated to make the claimed combination, Applicants are under no obligation to establish surprising or unexpected results to establish the non-obviousness of the present invention.

Moreover, Applicants note that contrary to the Examiner's statement, the specification provides advantages of using a tobacco chitinase hinge region as a linker. For example, page 16, lines 13-23 of the specification states that when a short anti-bacterial peptide, such as Sarcotoxin 1a, is expressed as a fusion protein to increase stability, the presence of the tobacco chitinase hinge region between the anti-bacterial peptide and a second protein would prevent steric hindrance of the peptide. Accordingly, the present specification provides advantages of using the tobacco chitinase hinge region in the present invention, and these advantages are sufficient to support patentability of these embodiments of the invention.

During the telephone interview on October 18, 1999, Applicants were also advised by the Examiner to submit a declaration discussing the unexpected results obtained with a construct such as those recited in new claims 32-40, wherein an inducible promoter and a constitutive promoter are positioned adjacent to each other. The Examiner stated that the advantages obtained with such a construct are prophetic and not actually shown. Moreover, it was understood by the undersigned that even if unexpected results are actually shown, the claims should be limited to the particular promoters used in the construct, namely, the tobacco PR-1a promoter and the CaMV 35S promoter.

Applicants respectfully disagree. As noted above, neither Jaynes *et al.* nor Broekaert *et al.* teach or suggest a transgenic plant comprising an expression vector as recited in claims 32-40. Therefore, claims 32-40 are not anticipated by or obvious over the cited references. Moreover, the present specification provides surprising results. It was well-known in the art that a gene operably linked to an inducible promoter is not expressed, unless an inducing agent is added. For example, the inducible nature of PR1a promoter is shown in Yamakawa *et al.*, *Plant Physiol.*, 118:1213-1222 (1998). This construct contains the tobacco PR1a-GUS construct, but does not contain a constitutive promoter, such as the CaMV 35S promoter. *See*, the "Analysis of PR-1 Gene Expression" section on page 1214. As shown in Figure 5, in the absence of an inducing chemical such as SA (salicylic acid) or Spm (spermine), almost no GUS activity is observed. By contrast, the embodiments of the invention containing an inducible PR1a promoter adjacent to a constitutive CaMV 35S promoter constitutively expresses a protein, irrespective of whether or not an inducing agent was added. *See, e.g.*, Figures 8 and 9 and page 25, lines 3-22 of the specification. These results are truly surprising, especially in light of Yamakawa *et al.* that shows the inducible nature of the PR1a promoter.

Furthermore, Applicants submit that it would be understood by those skilled in the art that similar effects could be obtained with other sets of inducible and constitutive promoters. As of the instant filing date, various inducible and constitutive plant promoters were well known in the art. For example, inducible promoters are reported in, *e.g.*, Balandin *et al.*, *Plant Mol. Biol.*, 27:1197-204 (1995), Fisscher *et al.*, *Plant Mol. Biol.*, 26:873-86 (1994), Carrasco *et al.*, *Plant Mol. Biol.*, 21:1-15 (1993), *etc.* Also, constitutive promoters are reported in, *e.g.*, McElroy *et al.*, *Plant Cell* 2:163-171 (1990), Christensen *et al.*, *Plant Mol. Biol.* 18:675-689 (1992), Conci *et al.*, *Ann. Phytopath. Soc. Japan* 59:432-437 (1993), *etc.* In light of this, one of skill in the art would have been able to select a suitable set of inducible and constitutive promoters without undue experimentation to prepare expression vectors as recited in claim 21 with the above-mentioned advantages. Thus, the Examiner's requirement to limit certain embodiments of the claims to the tobacco PR-1a promoter and the CaMV 35S promoter is unduly restrictive.

For the foregoing reasons, the claimed invention is enabled by the disclosure of the subject application, and at the same time, the claimed invention is novel and non-obvious over the prior art of record.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested. If a telephone conference would expedite prosecution of this application, the Examiner is invited to telephone the undersigned at (415) 576-0200.

Respectfully submitted,

Kathleen L. Choi
Reg. No. 43,433

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, 8th Floor
San Francisco, California 94111-3834
Tel: (415) 576-0200
Fax: (415) 576-0300
KLC
SF 1052807 v1